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## BIOLOGICAL BULLETIN

## IDENTICAL TWINS IN CATTLE? 1

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The phenomenon of a sterile, female-appearing animal resulting as a co-twin to a normal male has called attention to the problem, Do identical twins occur in cattle?

Three lines of evidence are available in answer to this question. The first consists of anatomical evidence, the ovaries of a twin-producing cow during pregnancy. This is the most critical and most desired. The second consists in the study of the normal sex ratios occurring in multiple births of cattle in comparison with those occurring in species where identical twins are produced. The third method consists in a comparison of the color markings of twins of the same or opposite sex.

In his paper on the freemartin, F. R. Lillie² collects data on the condition of the ovaries of cows producing twins. He showed in 22 cases where both ovaries were obtained corpus luteum were present in each. In 11 cases where only one ovary was obtained 9 had corpus luteum in that ovary. The two pairs of twins whose mothers lacked corpora lutea in the remaining ovary were of the same sex, one pair being males, the other pair females. These last two pair of twins may be identical or monozygotic twins. This appears doubtful, for as the data of Cole presented later will show, the proportion of single ovaries ovulating twice when the other one does not ovulate should not be very far from the proportion indicated by Lillie's data, providing, of course, that one ovary may ovulate twice when the other does not ovulate at all.

Lillie furnishes other data on the sex ratio of intrauterine twins

<sup>&</sup>lt;sup>1</sup> Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 141.

<sup>&</sup>lt;sup>2</sup> Lillie, Frank R., 1917, "The Freemartin; A Study of the Action of Sex Hormones in the Foetal Life of Cattle," in *Jour. Expt. Zoöl.*, Vol. 23, p. 371-422.

beside that indicated above. The ratios which he reports for all his data are 19 male, 24 male and female, and 11 female, the methods adopted being such as to insure a random sample of the general population of cattle twins. Cole<sup>1</sup> has also furnished data on the sex ratios of cattle twins which he obtained from cattle breeders' records of 303 twins. The ratios of these cattle twins were 43 male, 165 male and female, and 88 females. Seven cases of triplets were also reported. Besides these statistics the Maine Agricultural Experiment Station has collected data on the frequency of the different sexes of twins as they occur in Maine.2 These twin births were born to services for which this station had the service records. All services were traced in case the birth record did not appear at the expected time and either the proper birth record obtained, the cow found barren or the cow killed before parturition. These records should form a very homogeneous set of data in which the sex of the twins was governed only by chance. The data from these results show 5 males, 16 male and female, and 5 females. The University of Maine Animal Industry Department has alwäys kept records of its herds since it was established. These records should be a homogeneous set of data wherein only chance was acting on the sex of the twin births as the service dates and the disposal of all animals are known. The ratio of the sex of these twin births in these data are 4 males, 4 male and female, and 3 females. These data summarized are shown in Table I.

Authority.	Males.	Male and Female.	Females.	Р.	P for a 1:2:1 Ratio.
A. Cole	19	165 24 20	88 11 8	AB. 001 BC. 55 AC. 25	.045 .29 .92

<sup>&</sup>lt;sup>1</sup> Cole, L. J., 1916, "Twinning in Cattle with Special Reference to the Freemartin," Science, N. S., Vol. 43, p. 177.

<sup>&</sup>lt;sup>2</sup> The coöperative data mentioned were collected by Dr. Raymond Pearl while at this Station. The records for the college herd were obtained from the Animal Husbandry Department by the author.

The comparison of the three frequency distributions is made under P. The data of Cole and Lillie clearly do not correspond, the male class in Cole's frequency distribution being proportionately small, whereas the male class in Lillie's data is proportionately large. The data of Lillie and this paper correspond rather well. Here, again, the chief trouble is in the proportional differences of the male classes. The data of Cole and this paper are not so very far different, the great discrepancy being in the male class. The data of this paper are clearly about half way between those of Cole and Lillie in the proportion of sex. Cole's data were obtained from breeders. It is well known that the breeders register only about one half as many males as females in their herd books. Without question Cole would not knowingly have taken data so loaded. It is conceivable, however, that the breeders from whom he obtained his data might have been influenced by this proportion.

If identical twins are lacking in cattle, the proportion of births should be 1:2:1, providing no disturbances like lethal factors exist in the stock. The comparison of the frequency distributions to this proportion are given in the second column of P for Table I. No excessive deviations from this proportion exist. The poorest approach to expectation is the distribution presented by Cole. Here, judging from the male and female twins and the female twins, the male twins should be more frequent. This evidence is opposed to that of expectation on the basis of the production of identical twins.1 Thus in human twin births, where identical twins are produced, the proportions of sex are: males 234,497, male and female 264,098, females 219.312, according to Nichols.2 This proportion corresponds to a ratio of 1.07:1.20:1. In species where identical twins are produced it is clear that the like sex should be produced more frequently than the unlike sex of twins, whereas in Cole's data the like sex, the males in particular, are in defect. The proportion of twins as given by Lillie's data is more nearly in accord with the expectation of some identical twins. However, it will be noted that the male class is in excess and the

<sup>1</sup> The explanation of Cole for this difference will be taken up later.

<sup>&</sup>lt;sup>2</sup> Nichols, J. B., Memoirs of the American Anthropological Ass., I (1907), also in Newman, H. H., "The Biology of Twins," Univ. Chicago Science Series.

female class in defect of its expected ratio. The agreement between the proportions of twins for these data and the proportions of twins on the theory that they are distributed in accord with those of human twins, where identical twins are known to exist, shows a slightly poorer agreement (P = .24) than does the theory of 1:2:1. The data of this paper clearly show a distribution of 1:2:1, indicating that identical twins are seldom produced in cattle.

Independent confirmation of this hypothesis may be obtained. The American Jersey Cattle Club describes the animals registered. From the Herd Books of this Association 749 twin females, 168 twin males and 207 male and female were taken for a comparison of their color markings. These twins have been taken and their white markings, tongue color and switch color recorded. The by-laws of this Society in 1913 do not mention any requirement to indicate that the female twin can not be registered before she is proved a breeder. Besides these, 523 pairs of females, the offspring of the same sire and dam, have been tabulated for these colors. These last were found from the Registry of Merit tables in conjunction with the Herd Books. Table II. shows the distribution for these data.

TABLE II.

	Female Twins.		Male Twins.		Male & Fe- male Twins.			
	Ab- so- lute.	Per- cent- age.	Ab- so- lute.	Per- cent- age.	Ab- so- lute.	Per- cent- age.	Ab- so- lute.	Per- cent- age.
Alike in all particulars	449	59.95	1 =	61.31	117	56.52	_	57.36
One body mark different	22	2.94	8	4.76	5	2.42	19	3.63
Many body marks different	43	5.74	7	4.17	15	7.25	35	6.69
Tongue color different	36	4.81	I 2	7.14		7.25	33	6.31
Switch color different	20	2.67	7	4.17	8	3.86	12	2.29
Tongue and body color different	22	2.94	3	1.79	4	1.93	16	3.06
Switch and body color different Switch and tongue color differ-	57	7.61	10	5.95	12	5.80	20	3.82
ent	25	3.34	. 5	2.98	7	3.38	15	2.87
Different in all particulars	75	10.01	13	7.74	,	11.59	73	13.96
Total	747		168		207	35	523	

Table II. shows that the percentage of cows which are alike in

their body markings, tongue color and switch color are approximately the same for the four groups of cattle. The greatest difference is between the twins which are the males and the twins which are the male and female. Here the difference favors the like sex twins by about 5 per cent. It will be noted that the like sex twins resemble each other between 3.43 and 4.87 per hundred more than do the twins which are male and female. If the like sex twins are compared for resemblances with the sister, sister combination of column five, the difference becomes less. These facts all point to not more than a low percentage of identical twins in cattle if they exist at all.

If the differences are compared and their significance determined, it will be seen that none of the combinations, female twins, male twins, male and female twins or sister and sister, are significantly different. In fact, the differences are at no time greater than two times the probable error of such difference. If the whole distributions are compared by the  $\chi^2$  method, it is seen that they resemble each other closely. Such being the case, it can not be argued that twins of identical markings, tongue color or switch color exist in cattle.

In the Armadillo,¹ Dasypus novemcinctus, when the 4 offspring are known to be of monozygotic origin it has been shown that the degree of resemblance between the members of the quadruplet is very high. This resemblance is nearly twice as great as that which exists between brothers of known dizygotic origin. It is consequently fair to assume that the production of identical twins, in the sense that they arise from a single egg, will increase the proportion of identical offspring in like sex twins as compared with twins of unlike sex, or sister and sister combination. The above data have shown that the proportion of like sexed twins do not differ appreciably in the proportions which resemble each other from those which are of unlike sex or of sister, sister combination. Such being the case, it follows that identical twins (monozygotic) are rarely or never produced in cattle.

The importance of this conclusion as related to the freemartin problem has previously been discussed by F. R. Lillie<sup>1</sup> and need only be mentioned here. Hart, Cole and others have assumed that

<sup>1</sup> Loc. cit.

the freemartin is an undifferentiated male formed from the division of a single male-producing egg. Clearly, on this basis, these animals should be identical in appearance and the identical classes in the groups female twins, male twins and male and female twins should be large compared with those of the sister, sister class. As this is not so, it becomes doubtful if the hypothesis adopted by them will hold for the origin of the freemartin.